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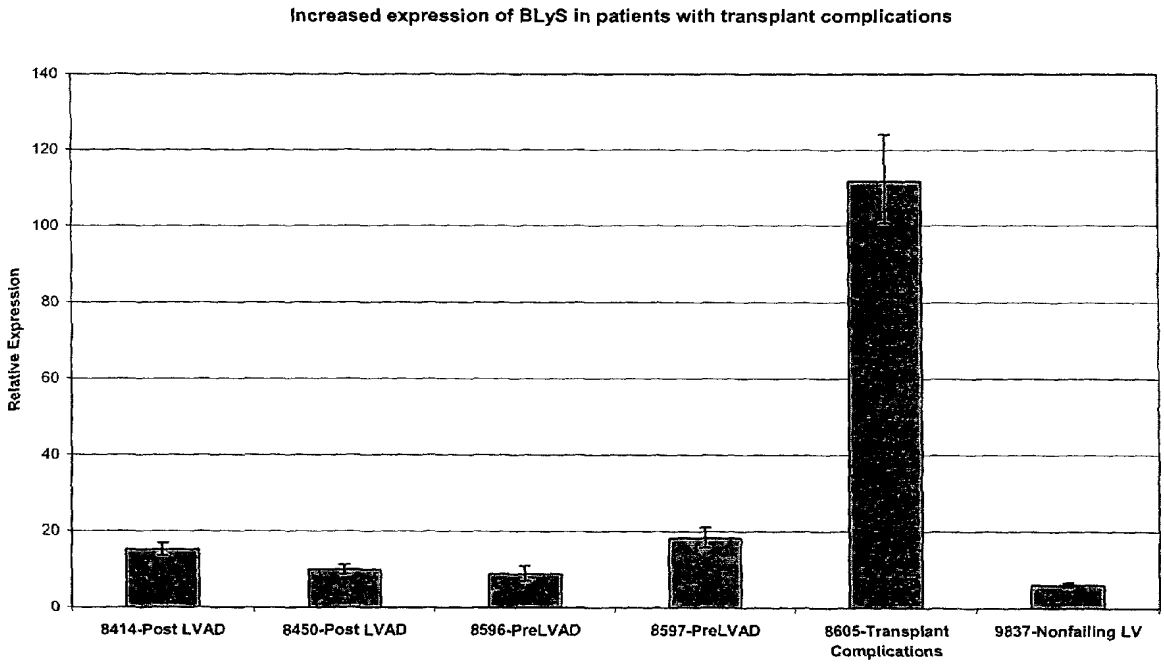
(54) Title: METHODS FOR THE DIAGNOSIS AND TREATMENT OF CARDIAC TISSUE REJECTION



(57) Abstract: Methods are provided for diagnosing and detecting cardiac rejection in transplant patients. Elevated levels of BlyS are found to be closely associated with cardiac rejection in transplant patients. Methods and compositions for blocking the expression and/or activity of BlyS are described that inhibit or prevent tissue rejection in cardiac transplant patients.

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METHODS FOR THE DIAGNOSIS AND TREATMENT OF CARDIAC TISSUE REJECTION

BACKGROUND OF THE INVENTION

The present invention provides methods for the diagnosis and treatment of cardiac tissue rejection in heart transplant recipients. Specifically, the invention provides methods for measuring levels of expression of a marker gene in a patient, where elevated expression of that gene indicates that the patient is rejecting the transplanted heart. The invention also provides methods for inhibiting the expression and/or the activity of the marker gene, thereby suppressing the rejection of the transplanted heart.

Heart transplant patients experience on average two episodes of rejection of their transplanted hearts during the first year after the transplant, with the first 3 months being the most critical. It is apparent, therefore, that such patients need to be monitored to detect signs of transplant rejection at the earliest possible stage.

The most common way to monitor the presence or absence of tissue rejection is to obtain a biopsy sample of the heart which then is assayed for the presence and number of T-lymphocytes. An alternative method is to assay the level of HLA-specific antibodies in the patient's blood after transplantation. However, both of these methods rely on the presence of an active immune response against the transplanted cardiac tissue, whereas it would be preferable if the potential for rejection could be assessed prior to development of this immune response.

It is apparent therefore, that new methods for diagnosing cardiac transplant rejection at an early stage are greatly to be desired. Moreover, it also is apparent that new methods for controlling cardiac rejection in transplant patients are highly desirable.

SUMMARY OF THE INVENTION

It is therefore an object of this invention to provide improved methods for diagnosing or predicting rejection phenomena in cardiac transplant patients.

It is another object of this invention to provide methods and compositions for the prevention and treatment of rejection in cardiac transplant patients.

In accordance with the present invention, there is provided, in an embodiment, a method for assaying the expression of the protein BLyS (B-lymphocyte stimulator). In one embodiment, the BLyS expression is assayed at the nucleic acid level, by measurement of levels of BLyS-encoding mRNA in cardiac biopsy tissue. In another embodiment, levels of BLyS are assayed at the protein level by measuring concentration of BLyS in cardiac tissue or in peripheral blood. Methods suitable for assaying BLyS include nucleic acid microarray, qPCR and ELISA.

According to another embodiment of the invention, there are provided methods and compositions for inhibiting the expression and/or activity of BLyS and thereby inhibiting rejection in cardiac transplant patients. In one embodiment, BLyS expression is inhibited at the nucleic acid level using an antisense molecule that binds to BLyS-encoding mRNA and preventing translation of the message. In an alternative embodiment, mRNA translation is inhibited using a nucleic acid molecule that promotes triplex (triple helix) formation. BLyS-encoding mRNA may also be targeted using ribozymes or RNAi. In another embodiment, BLyS activity is inhibited at the protein level using an antibody, such as a human or humanized antibody, or other binding protein that binds to and inactivates BLyS. In yet another embodiment, BLyS activity is inhibited at the protein level using a soluble receptor molecule that sequesters BLyS.

In a further embodiment, the composition is administered together with an additional compound that suppresses cardiac rejection, such as cyclosporine, prednisone, azathioprine, tacrolimus or FK506, mycophenolate mofetil, OKT3, ATGAM or thymoglobulin.

Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE FIGURE

Figure 1 shows the increased expression of BLyS in patients having allograft transplant complications (column 8605-Transplant complications) versus patients pre- and post LVAD (left ventricular assist device) or with non-failing left ventricle (LV).

DETAILED DESCRIPTION

Methods are provided for diagnosing and detecting cardiac rejection in transplant patients. Specifically, the methods involve assaying expression of BLyS in those patients. Elevated levels of BLyS are found to be closely associated with cardiac rejection in transplant patients. In particular, assay of BLyS levels allows an early indication of graft rejection prior to the clinical observation of rejection, and permits early intervention to block rejection. Methods and compositions for blocking the expression and/or activity of BLyS may be used to inhibit or prevent tissue rejection in cardiac transplant patients.

BLyS is a member of the tumor necrosis factor (TNF) superfamily of proteins and stimulates proliferation and immunoglobulin production in plasma B cells. BLyS is also known as BAFF, TALL-1 and THANK. BLyS is a secreted protein expressed in activated monocytes and macrophages that induces B cell proliferation and differentiation. Moore *et al.*, "BLyS: Member of the Tumor Necrosis Factor Family and B Lymphocyte Stimulator". *Science*, 285:260-263 (1999). BLyS is expressed in a 285 amino acid membrane-bound form, and also as a soluble 152 amino acid protein. Upon activation, soluble BLyS is released and can bind to at least two B-cell specific receptors, known as TACI and BCMA. Gross *et al.*, *Nature* 404:995-9 (2000). TACI is an orphan TNF receptor homologue of unknown function. Binding of BLyS to TACI activates signaling by the transcription factors NF-kappa B and ELF-1.

Inhibition of BLyS activity in peripheral blood cells *in vitro* has been shown to block BLyS-induced NF-kappa B activation in B lymphoma cells and IgM production in peripheral blood B cells. *In vivo* treatment of immunized mice with TACI-Fc has been shown to inhibit production of antigen-specific IgM and IgGI antibodies and abolish splenic germinal center (GC) formation. Yan *et al., Nat. Immunol* 1:37-41 (2000). It has been postulated that BLyS activity must play a critical role in the humoral immune response. *Id.* Membrane bound BLyS has the protein sequence:

MDDSTEREQSRLTSCLKKREEMKLKECVSILPRKESPSVRSSKD
GKLLAATLLLALLSCCLTVVSFYQVAALQGDLASLRAELQGHH
AEKLPAGAGAPKAGLEEAPAVTAGLKIFEPPAPGEGNSSQNSR
NKRAVQGPEETVTQDCLQLIADSETPTIQKGSYTFVPWLLSFKR
GSALEEKENKILVKETGYFFIYGQVLYTDKTYAMGHLIQRKKV
HVFGDELSLVTLFRCIQNMPETLPNNSCYSAGIAKLEEGDELQL
AIPRENAQISLDGDVTFFGALKLL

To identify genes that possible were involved in graft rejection, gene expression was measured in heart failure myocardial tissues and nonfailing myocardial tissues archived from 400 patients. The gene expression was studied using the GeneChip® DNA microarray platform (Affymetrix, Santa Clara, CA). The Human Genome U95 Set was used which contains a comprehensive transcript coverage of the human genome. Cardiac tissue biopsy samples were obtained, and mRNA was prepared according to the manufacturer's directions. The results obtained confirmed changes in regulation of known genes previously demonstrated in the literature and also identified the involvement of BLyS in the rejection of transplanted hearts. A general trend of elevated BLyS expression was observed in failing transplanted hearts when compared to non-failing (non-rejecting) heart tissue. In particular, levels of BLyS expression were at least about two-fold higher than in non-failing tissue, and in some cases were up to about ten-fold higher than in non-failing tissue.

Methods for detecting levels of BLyS expression

This observation of increased BLyS expression in transplanted cardiac tissue that is being rejected provides a new and powerful method for an early diagnosis of graft rejection in transplant patients by measuring BLyS expression levels. Methods for determining protein expression levels are well known in the art, and can be carried out at either the nucleic acid or protein level.

For example, BLyS-encoding mRNA can be obtained from a cardiac tissue biopsy of a patient under study and quantified using methods that are well known in the art. BLyS-encoding mRNA also can be obtained from peripheral blood lymphocytes and levels of BLyS expression determined.

By way of example, methods of quantitative polymerase chain reaction (QPCR) are well known and can be carried out using kits and methods that

are commercially available from, for example, Applied BioSystems (Foster City, CA) and Stratagene (La Jolla, CA). See also Kochanowski, "Quantitative PCR Protocols," Humana Press, 1999. Alternatively, quantitative analysis can also be carried out using nucleic acid arrays by methods that are well known in the art, for example, using arrays provided by Affymetrix Inc (Santa Clara, CA). Alternative methods for measuring gene expression levels are well known, for example by Northern blot, nuclease protection, quantitative RT-PCR, and differential display methods such as SAGE analysis (see Velculescu *et al., Science* 270, 484-487 (1995)). The skilled artisan will recognize that other methods are available, and that new methods for measuring levels of gene expression that are not presently known will also be suitable for use in the present invention.

Methods of assaying gene expression at the protein level are also well known in the art, for example, using antibody-based ELISA analysis. Antibodies against BLyS for use in ELISA analysis can be obtained using well known methods, including by hybridoma-based methodologies, or by screening of phage display libraries such as those available from Dyax (Cambridge, MA), MorphoSys (Martinsried, Germany), Biosite (San Diego CA) and Cambridge Antibody Technology (Cambridge UK). Antibodies that bind to BLyS are known and are described, for example, in WO 02/02641, the disclosure of which is hereby incorporated by reference in its entirety. Other polypeptides that bind to BLyS and that are suitable for measuring BLyS expression levels are described in WO 02/16411, the disclosure of which is also hereby incorporated by reference in its entirety.

BLyS levels can be assayed in cardiac tissue biopsy samples or in peripheral blood. In each case the levels of BLyS in the patient under study are compared to a reference level either from a patient that has not undergone heart transplant or from a patient with a transplanted heart that shows no indication of being rejected. Reference levels can be obtained from a number

of healthy patients to establish a baseline level of BLyS in healthy cardiac tissue or in circulating blood. Methods for assaying BLyS levels in patients have also been reported: Zhang *et al.*, *J. Immunol.* 166:6-10 (2001), which is hereby incorporated by reference in its entirety.

In the context of the present invention, a statistically significant increase in BLyS expression levels in a transplant patient compared to a baseline is considered indicative of transplant rejection. More particularly, an approximately 1.5-2 fold or higher increase in BLyS expression over baseline is considered to be diagnostic of rejection. Higher levels of BLyS expression are indicative of a greater likelihood of rejection and/or a later stage of rejection.

Methods for inhibiting BLyS expression

The present invention also provides methods for ameliorating, reducing, inhibiting and./or preventing cardiac transplant rejection by inhibiting the expression and/or activity of BLyS. Methods of inhibiting protein expression and/or function in a patient are well known in the art. Thus, BLyS expression can be inhibited at the nucleic acid level using antisense reagents using well known methods. Suitable reagents are described, for example, in US Patent Nos. 5,989,912 and 5,849,902, which are hereby incorporated by reference in their entirety. Other methods for designing and manufacturing antisense reagents that are stable in the human body are well known in the art. The anti-sense reagent may be antisense oligonucleotides, particularly synthetic antisense oligonucleotides having chemical modifications from native nucleic acids, or nucleic acid constructs that express such anti-sense molecules as RNA. The antisense sequence is complementary to the mRNA of the targeted gene, and inhibits expression of the targeted gene products. Antisense molecules inhibit gene expression through various possible mechanisms, *e.g.*, by reducing the amount of mRNA

available for translation, through activation of RNAseH or steric hindrance. One or a combination of antisense molecules may be administered, where a combination may comprise multiple different sequences.

Antisense molecules may be produced by expression of all or a part of the target gene sequence in an appropriate vector, where the transcriptional initiation is oriented such that an antisense strand is produced as an RNA molecule. Alternatively, the antisense molecule is a synthetic oligonucleotide. Antisense oligonucleotides will generally be at least about seven, usually at least about twelve to fourteen, and more usually at least about twenty nucleotides in length. Typical antisense oligonucleotides are usually not more than about five-hundred, more usually not more than about fifty, and even more usually not more than about thirty-five nucleotides in length, where the length is governed by efficiency of inhibition, specificity, including absence of cross-reactivity, and the like. It has been found that short oligonucleotides, of from seven to eight bases in length, can be strong and selective inhibitors of gene expression (see Wagner *et al.* (1996) Nat. Biotech. 14, 840-844).

A specific region or regions of the BLyS mRNA sequence is chosen to be complemented by the antisense sequence. Selection of a specific sequence for the oligonucleotide may use an empirical method, where several candidate sequences are assayed for inhibition of expression of the target gene in an *in vitro* or animal model. A combination of sequences may also be used, where several regions of the mRNA sequence are selected for antisense complementation. Computer-aided methods for designing or selecting appropriate antisense oligonucleotides are known in the art.

Antisense oligonucleotides may be chemically synthesized by methods known in the art (see Wagner *et al.* (1996) Nat. Biotech. 14, 840-844). Preferred oligonucleotides are chemically modified from the native phosphodiester structure, in order to increase their intracellular stability and

binding affinity. Many such modifications have been described in the literature, which alter the chemistry of the backbone, sugars or heterocyclic bases.

Alternatively, gene expression can be inhibited using methods involving triplex-forming oligonucleotides such as those described in US Patent No. 5,650,316 and references described therein, which methods are hereby incorporated by reference in their entirety. Another method for blocking BLyS expression is via use of ribozymes. Methods for designing stabilized ribozymes that may be used to target specific mRNA *in vivo* are well known in the art. See, for example, Intracellular Ribozyme Applications:Principles and Protocols (Rossi and Couture, Eds. Horizon Scientific Press, 1999). Yet another method for inhibiting BLyS expression is via use of RNA interference (RNAi) technology via use of a homologous double stranded RNA (dsRNA) that specifically targets BLyS mRNA. See, for example, WO 02/44321 and WO 01/75164, which are hereby incorporated by reference in their entirety.

BLyS activity can also be blocked at the protein level using anti-BLyS antibodies, which are known in the art. Thus, inhibitory human antibodies that bind to and inhibit BLyS activity are known (see WO 02/02641 *supra*), or can be obtained from phage display libraries such as those described *supra*. Alternatively, human anti-BLyS antibodies can be obtained using xenomouse technology such as that available from Abgenix (Fremont, CA) and Medarex (Princeton, NJ). In addition, murine anti-BLyS antibodies obtained via conventional hybridoma techniques can be humanized using methods that are well known in the art. See, for example, U.S. Patents No. 5,693,762 and 5,585,089, which are hereby incorporated by reference in their entirety.

In another method, BLyS activity can be blocked by administering a soluble form of a BLyS binding protein such as a receptor. This approach has been shown to be effective in mice using a TACI-Fc fusion protein. See

Marsters *et al., Curr. Biol.* **10**: 785-8, which is hereby incorporated by reference in its entirety. In humans, this method would use a human BLyS receptor such as TACI in a suitable form, such as in a fusion protein with a human Fc fragment. BLyS-binding polypeptides such as those described in WO 02/16411 *supra*, may also be used.

The present invention further provides compositions containing one or more agents that modulate BLyS expression or protein activity. While individual needs vary, determination of optimal ranges of effective amounts of each component is within the skill of the art. Typical dosages comprise about 0.10 to 100.0 mg/kg body weight. The preferred dosages comprise about 0.10 to 10.0 mg/kg body weight. The most preferred dosages comprise about 0.10 to 1.0 mg/kg body weight.

In addition to the pharmacologically active agent, the pharmaceutical compositions may contain suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically for delivery to the site of action. Suitable formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form, for example, water-soluble salts. In addition, suspensions of the active compounds as appropriate oily injection suspensions may be administered. Suitable lipophilic solvents or vehicles include fatty oils, for example, sesame oil, or synthetic fatty acid esters, for example, ethyl oleate or triglycerides. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension include, for example, sodium carboxymethyl cellulose, sorbitol and/or dextran. Optionally, the suspension may also contain stabilizers. Liposomes can also be used to encapsulate the agent for delivery into the cell.

The pharmaceutical formulation for systemic administration according to the invention may be formulated for enteral, parenteral or topical

administration. Indeed, all three types of formulations may be used simultaneously to achieve systemic administration of the active ingredient. Suitable formulations for oral administration include hard or soft gelatin capsules, pills, tablets, including coated tablets, elixirs, suspensions, syrups or inhalations and controlled release forms thereof.

In practicing the methods of this invention, the pharmaceutical compositions may be used alone or in combination, or in combination with other therapeutic or diagnostic agents. In certain preferred embodiments, the compositions may be co-administered along with other compounds typically prescribed for treating or preventing cardiac rejection, for example, these conditions according to generally accepted medical practice, such as cyclosporine (Neoral, Sandimmune), prednisone (Novo Prednisone, Apo Prednisone), azathioprine (Imuran), tacrolimus or FK506 (Prograf), mycophenolate mofetil (CellCept), OKT3 (Muromorab CO3, Orthoclone), ATGAM (equine polyclonal antibodies against human thymocytes) and thymoglobulin. The compounds of this invention can be utilized *in vivo*, ordinarily in mammals, such as humans.

The present invention, thus generally described, will be understood more readily by reference to the following examples, which are provided by way of illustration and are not intended to be limiting of the present invention.

Example 1: Microarray analysis

RNA Extraction

Total RNA from biopsy specimens is isolated using Trizol™ (Invitrogen, Carlsbad, CA) according to the manufacturers protocol.

Target Preparation and DNA Microarray Hybridizations

mRNA is reverse transcribed using an HPLC purified oligo-dT primer with T7 sequence attached. The RNA and the primer is incubated at 70°C for

10 min. and put on ice. First strand cDNA buffer, DTT (10 mM final conc.), and dNTP mix is added and incubated for 2 min. at 37°C. Reverse transcriptase is added at 500 units per reaction to a total volume of 20 ml. The first strand reaction mix is incubated at 37°C for 1 hour and put on ice. For second strand synthesis, second strand reaction buffer, dNTPs (200 mM each) DNA ligase (10 units) DNA Polymerase (40 units), and RNase H (2 units) are added (all final concentrations). The volume is adjusted to 150 ml with DEPC-treated water. The second strand reaction mix is incubated at 16°C for 2 hours. 10 units of T4 polymerase are be added for an addition 5 min. of incubation (16°C) to fill in overhangs. The reaction is stopped with 10 ml of 0.5M EDTA and stored at –20°C. Double-stranded cDNA is cleaned up by phenol/chloroform extraction using phase lock gel to allow a more complete recovery of the sample. Nucleic acids are precipitated with ethanol. After second strand synthesis, an in vitro transcription and biotin-labeling of cRNA target are performed using the ENZO BioArray High Yield RNA Transcript Labeling Kit (ENZO Biochem, Inc., New York, NY) according to the manufacturers protocol. The labeled cRNA product is purified using RNeasy spin columns from QIAGEN according to the manufacturers protocol and ethanol-precipitated. cRNA yield is quantified by spectrophotometric analysis at 260 nm and 280 nm and run on an agarose gel to determine the size distribution of the labeled transcripts. To increase hybridization efficiency, the cRNA is fragmented to sizes of approximately 100 basepairs in length prior to the hybridization to DNA microarrays. Fragmentation is performed by heating the cRNA for 10 to 45 min. at 94°C in fragmentation buffer (40 mM Tris-acetate, pH 8.1, 100 mM KOAc, 30 mM MgOAc) and controlled on an agarose gel. Fragmented cRNA Test hybridization is performed using a GeneChip® Hybridization Oven (Affymetrix) on a Test 1 or a Test 2 probe array to ensure the quality of the target prior to exposing it to the GeneChip® DNA microarray. After hybridization, the GeneChip®

DNA microarrays are stained with a streptavidin-conjugate (Molecular Probes). Hybridization signals are detected using an HP GeneArray™ Scanner.

Verification of Gene Expression by Real-Time, Quantitative Reverse-Transcription PCR (Q-RT-PCR)

BLyS mRNA upregulation may be further verified and quantified by qRT-PCR analysis using an ABI PRISM 7700 Sequence Detection System. The relative quantitation of gene expression was analyzed, using 18S rRNA as endogenous control. One microgram of total RNA was reverse transcribed using random hexamers and the TaqMan Reverse Transcription Reagents Kit (Perkin Elmer) following the manufacturer's protocols. The reverse transcription was carried out at 48°C for 30 min. The cDNA was then amplified using TaqMan PCR master mix containing AmpEraseUNG, dNTP, AmpliTaq Gold, primers, and SYBR dye according to the manufacturer's protocols under the universal cycling conditions of 2 min. at 50°C, 10 min. at 95°C, followed by 40 cycles of 15 sec at 95°C, and 1 min. at 60°C. Standard curves for growth of signal for both endogenous control and the target gene were constructed and used to calculate the amount of target mRNA present in the original sample using standard methods. This technique has been widely used to characterize gene expression.

Results:

As shown in the Table below, 25 Affymetrix probe-specific target sequences with corresponding genes that were validated by standard protocols . Q-RT-PCR probes were selected according to either: 1.) Demonstrated up/down-regulation as determined by Affymetrix microarray and GeneExpress 2000™ analysis (Gene Logic, Gaithersburg, MD); or 2.) Demonstration of no apparent regulation by the above described analysis. Probe number 24 was designed according to the SIF sequence published by

Affymetrix for Homo sapiens tumor necrosis factor (ligand) superfamily, member 13b (BLyS). Figure 1 illustrates data that unequivocally confirms a greater than 5-fold up regulation of BLyS gene expression in human left ventricular myocardial tissue from patients experiencing complications of allograft transplantation, as compared to nonfailing left ventricular myocardium. "Complications" are defined as the pathological and histological identification of focal and/or diffuse lymphocyte infiltration and corresponding myocardial tissue necrosis. These data demonstrate the accuracy of Affymetrix microarray analysis for the above described human tissue samples. Importantly, BLyS gene expression did not change significantly in left ventricular tissue samples from patients undergoing left ventricular assist device (LVAD) implantation to support function of patients' diseased native (natural) hearts. This observation shows the specificity of BLyS up regulation in allograft, tissue-specific rejection since implantation of artificial devices, such as the LVAD, can readily induce host immune responses. Immune responses to the implantation of an artificial device are distinct in that up regulation of immunoglobulin-specific genes are observed in the absence of any change in BLyS expression.

Table: Validation Probes and Gene Targets

No.	Expression or AffyChip® Fragment ID	Fragment Name (Affy Element)	GenBank Accession No.	Name of Gene (if known)
1	104580	35391_at	AB009671	a disintegrin and metalloproteinase domain 22
2	104736	35906_at	L29339	solute carrier family 5 (sodium/glucose cotransporter), member 1
3	105527	38494_at	S65583	acrosomal vesicle protein 1
4	105731	38937_at	AF022152	adaptor-related protein complex 3, beta 2 subunit
5	105769	39215_at	M25296	natriuretic peptide precursor B
6	106543	41426_at	U38864	zinc finger protein 212

7	109121	39791_at	M23114	ATPase, Ca++ transporting, cardiac muscle, slow twitch 2
8	110237	34375_at	M28225	small inducible cytokine A2 (monocyte chemotactic protein 1, homologous to mouse Sig-je)
9	109305	40454_at	NM_005245	FAT Tumor Suppressor Gene
10	109399	40788_at	NM_001625	Adenylate Kinase 2
11	112638	32521_at	NM_003012	Homo sapiens secreted frizzled-related protein 1
12	113059	1893_s_at	NM_000125	Homo sapiens estrogen receptor 1 (ESR1), mRNA
13	118715	44105_at	NM_016084	Homo sapiens RAS, dexamethasone-induced 1 (RASD1), mRNA
14	102541	74622_at	BC026215	Syntrophin
15	119803	48809_s_at	AF155100	ZF3 gene; zinc-finger protein
16	122669	47774_at	NM_00006587	Corin
17	131134	64191_at	BC022524	Homo sapiens fibroblast growth factor 12B (FGF12B), mRNA
18	143881	74815_at	NM_000582	Homo sapiens secreted phosphoprotein 1
19	102381	31400_at	XM_044625	Diacylglycerol Kinase
20	102514	31533_s_at	XM_052127	KCND3 (K^+ Channel)
21	156838	75582_at	NM_032507	Cerebral Protein-4
22	163889	38734_at	BC005269	Homo sapiens phospholamban (PLN), mRNA
23	164017	89860_at	XM_011540	Homo sapiens ectonucleotide pyrophosphatase/phosphodiesterase 3
24	136482	53773_at	NM_006573	**Homo sapiens tumor necrosis factor (ligand) superfamily, member 13b (BLyS)**
25	108225	37219_at	NM_002416	Homo sapiens monokine induced by gamma interferon (MIG), mRNA

What is claimed is:

1. A method of diagnosing rejection in a cardiac transplant patient, comprising assaying the level of expression of BLyS in a sample obtained from said patient.

2. The method according to claim 1, wherein said level is compared against a reference level, and wherein an increased level of BLyS expression is indicative of cardiac rejection.

3. A method of predicting the likelihood that a cardiac transplant patient will experience rejection of the transplanted cardiac tissue, comprising assaying the level of expression of BLyS in a sample obtained from said patient.

4. The method according to claim 3, wherein said level is compared against a reference level, and wherein an increased level of BLyS expression is predictive of cardiac rejection.

5. The method according to any of claims 1-4, wherein said assay measures BLyS mRNA levels.

6. The method according to any of claims 1-4, wherein said assay measures BLyS protein levels.

7. The method according to any of claims 1-6, wherein said assay uses cardiac biopsy tissue from said patient.

8. The method according to any of claims 1-6, wherein said assay uses blood obtained from said patient.

9. The method according to any of claims 1-5 or 7-8 wherein said assay uses a nucleic acid array to measure BLyS levels.

10. The method according to any of claims 1-5 or 7-8 wherein said assay uses quantitative PCR to measure BLyS levels.

11. The method according to any of claims 1-4 or 6-8 wherein said assay uses an ELISA method to measure BLyS levels.

12. A method of inhibiting cardiac transplant rejection comprising administering to a patient suffering from said rejection an effective amount of a composition that inhibits BLyS activity.

13. The method according to claim 12, wherein said composition inhibits BLyS expression.

14. The method according to claim 12, wherein said composition inhibits BLyS protein activity.

15. The method according to claim 13 wherein said composition comprises an antisense oligonucleotide that binds to BLyS mRNA.

16. The method according to claim 13 wherein said composition comprises an oligonucleotide that binds to BLyS mRNA to form a triplex.

17. The method according to claim 13 wherein said composition comprises a ribozyme that cleaves BLyS mRNA.

18. The method according to claim 14 wherein said composition comprises an antibody that binds to BLyS protein.

19. The method according to claim 18 wherein said antibody is a human antibody.

20. The method according to claim 14 wherein said composition comprises a soluble BLyS receptor.

21. The method according to claim 20, wherein said receptor is TACI.

22. The method according to claim 12, wherein said composition is administered together with an additional compound that suppresses cardiac rejection.

23. The method according to claim 22, wherein said additional compound that suppresses cardiac rejection is selected from the group consisting of cyclosporine, prednisone, azathioprine, tacrolimus or FK506, mycophenolate mofetil, OKT3, ATGAM and Thymoglobulin

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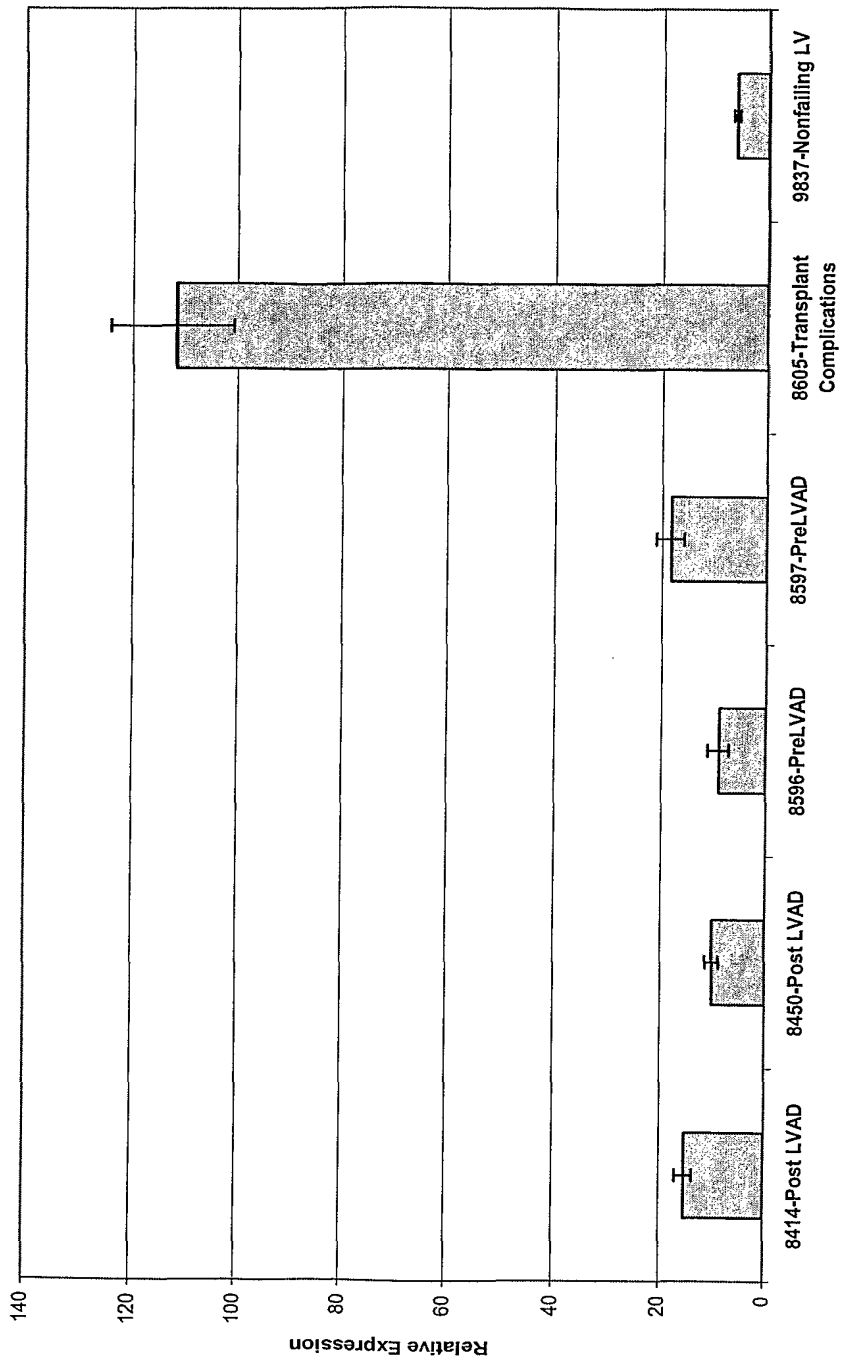

Figure 1: Increased expression of BLyS in patients with transplant complications